AMENDMENT

To: Examiner of the Patent Office

1. Identification of the International Application
PCT/JP2004/016717

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4. Item to be Amended: Description and Claims

5. Subject Matter of Amendment
(1)The Applicant removes the following description from the specification (corresponding to WO 2005/042370A2): page 7, lines 15-19; page 8, lines 23-25; page 25, portions of lines 5, 6, 26 and 27; page 32,

lines 12-15; and page 61, lines 8 and 9.

(2)The Applicant submits an amendment to amend claims 1, 14, 22, 28, 40, 42-48 and 70-72; delete claims 16, 17, and 25.

6. List of Attached Documents

(1)Replacement sheet of page 7, 8, 25, 32 and 61

(2)Replacement sheet of page 114-120, 121/1, 121/2, 126, and 127

13. The method according to Item 1, further comprising the step of removing blood cells.

14. A method for preparing a stem cell comprising:

A) obtaining material from liposuction; and

B) subjecting the material from liposuction to centrifugation to obtain a cell fraction without isolation of fat tissue.

15. The method according to Item 14, further comprising the step of subjecting the material to a condition where at least a portion of cells are separated from the material.

~~16. The method according to Item 15, wherein the condition is for degradation of extracellular matrices.~~

~~17. The method according to Item 15, said degradation of extracellular matrices is achieved by a collagenase.~~

18. The method according to Item 14, further comprising the step of removing supernatant in step B).

19. The method according to Item 14, further comprising the step of filtering the material from the step B).

20. The method according to Item 14, further comprising the step of removing blood cells.

21. The method according to Item 14 wherein the step of removing blood cells comprises adding a component of degrading blood cells.

22. A method for preparing a stem cell comprising:

i) obtaining material from liposuction;

ii) subjecting the material to a condition where at least a portion of cells are separated from the material, without isolation of fat tissue;

iii) subjecting the material to centrifugation;

iv) adding a component degrading blood cells to the material and agitating the material;

v) subjecting the material to centrifugation to obtain a pellet; and

vi) aspirating supernatant of the material from the pellet.

23. The method according to Item 22, wherein the step of subjecting the material to said condition comprises maintaining an aspirate from the liposuction.

24. The method according to Item 22, wherein said material from liposuction comprises an aspirate from liposuction and fat.

~~25. The method according to Item 22, wherein said condition in said step ii) comprises adding a collagenase.~~

26. The method according to Item 22, wherein the centrifugation in said step iii) is conducted at 400-1200 x g.

27. The method according to Item 22, wherein said component degrading blood cells comprises ammonium chloride and potassium bicarbonate.

cell, which has monopotency, multipotency, or totipotency. Stem cells can be differentiated in response to specific stimuli. Typically, stem cells can regenerate an injured tissue. Stem cells used herein may be, but are not limited to, ~~embryonic stem (ES) cells,~~ tissue stem cells (also called tissular stem cell, tissue-specific stem cell, or somatic stem cell), or other precursor cells. A stem cell may be an artificially produced cell (e.g., fusion cells, reprogrammed cells, or the like used herein) as long as it can have the above-described abilities. Embryonic stem cells are pluripotent stem cells derived from early embryos. An embryonic stem cell was first established in 1981, which has been applied to production of knockout mice since 1989. In 1998, a human embryonic stem cell was established, which is currently becoming available for regenerative medicine. Tissue stem cells have a relatively limited level of differentiation unlike embryonic stem cells. Tissue stem cells are present in tissues and have an undifferentiated intracellular structure. Tissue stem cells have a higher nucleus/cytoplasm ratio and have few intracellular organelles. Most tissue stem cells have pluripotency, a long cell cycle, and proliferative ability beyond the life of the individual. As used herein, stem cells may be ~~preferably embryonic stem cells, though~~ tissue stem cells ~~may also be~~ employed depending on the circumstance.

Tissue stem cells are separated into categories based on the sites from which the cells are derived, such as the dermal system, the digestive system, the bone marrow system, the nervous system, and

function and/or form in a multicellular organism. "Tissue" is typically an aggregate of cells of the same origin, but may be an aggregate of cells of different origins as long as the cells have the same function and/or form. Therefore, when stem cells of the present invention are used to regenerate tissue, the tissue may be composed of an aggregate of cells of two or more different origins. Typically, a tissue constitutes a part of an organ. Animal tissues are separated into epithelial tissue, connective tissue, muscular tissue, nervous tissue, and the like, on a morphological, functional, or developmental basis. ~~Plant tissues are roughly separated into meristematic tissue and permanent tissue according to the development stage of the cells constituting the tissue.~~ Alternatively, tissues may be separated into single tissues and composite tissues according to the type of cells constituting the tissue. Thus, tissues are separated into various categories. Any tissue may be herein intended as a target to be treated.

Any organ may be targeted by the present invention. A tissue or cell targeted by the present invention may be derived from any organ. As used herein, the term "organ" refers to a morphologically independent structure localized at a particular portion of an individual organism in which a certain function is performed. In multicellular organisms (e.g., animals, plants), an organ consists of several tissues spatially arranged in a particular manner, each tissue being composed of a number of cells. An example of such an organ includes an organ relating to the vascular system. In one embodiment, organs targeted by the present

The material from liposuction used in the present invention usually includes an aspirate from liposuction and fat, however, it was found that when treated according to the preset invention, the material contains many more stem cells than that found in an aspirate.

~~Preferably, said condition in said step ii) comprises adding a collagenase.~~

Preferably, the present method may further comprise the step of subjecting the material to said condition comprises maintaining an aspirate from the liposuction.

Preferably, the material from liposuction used in the present invention, may further comprises an aspirate from liposuction and fat.

In another embodiment, the centrifugation in said step iii) is conducted at 400-1200 x g. Usually 400 x g or 800 x g is used.

In another embodiment, said component degrading blood cells comprises ammonium chloride and potassium bicarbonate.

In another embodiment, said ammonium chloride is comprised in the component at 100 mM to 200 mM, preferably at about 155mM. In another embodiment, said potassium bicarbonate is comprised in the component at 5 mM to 20 mM, preferably about 10mM. Preferably, the combination of the two is advantageously used.

CLAIMS

What is claimed is:

1. (Amended) A method for preparing a stem cell, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction

C) subjecting the cell fraction to centrifugation by specific gravity; and

D) collecting a cell layer with lower specific gravity than that of erythrocytes.

2. The method according to Claim 1, wherein said aspirate from liposuction is prepared using saline or Ringer's solution.

3. The method according to Claim 1, wherein said centrifugation is conducted at a speed of a range equal to or less than 800 x g.

4. The method according to Claim 1, wherein said centrifugation is conducted at a speed of a range equal to or less than 400 x g.

5. The method according to Claim 1, wherein said centrifugation by specific gravity is conducted at a speed of a range between 370 x g and 1,100 x g.

6. The method according to Claim 1, wherein said centrifugation by specific gravity is conducted using medium which as a specific gravity of 1.076 to 1.078

g/ml at 20 degree Celsius.

7. The method according to Claim 1, wherein the medium of said centrifugation by specific gravity is selected from the group consisting of Ficoll, Percoll and sucrose.

8. The method according to Claim 7, wherein the medium of said centrifugation by specific gravity is Ficoll.

9. The method according to Claim 1, wherein the specific gravity of the collected cell layer is at a range of between 1.050 and 1.075.

10. The method according to Claim 1, wherein the collection of said cell layer is conducted using a pipette.

11. The method according to Claim 1, further comprising the step of culturing said cell layer in a medium containing components selected from the group consisting of DMEM, M199, MEM, HBSS, Ham's F12, BME, RPMI1640, MCDB104, MCDB153(KGM) and a mixture thereof.

12. The method according to Claim 1, wherein the centrifugation by specific gravity comprises density gradient centrifugation.

13. The method according to Claim 1, further comprising the step of removing blood cells.

14.(Amended) A method for preparing a stem cell,

without collagenase treatment, comprising:

A) obtaining material from liposuction; and

B) subjecting the material from liposuction to centrifugation to obtain a cell fraction without isolation of fat tissue.

15. The method according to Claim 14, further comprising the step of subjecting the material to a condition where at least a portion of cells are separated from the material.

(Cancelled)[~~16. The Method according to Claim 15, wherein the condition is for degradation of extracellular matrices.~~]

(Cancelled)[~~17. The method according to Claim 15, said degradation of extracellular matrices is achieved by a collagenase.~~]

18. The method according to Claim 14, further comprising the step of removing supernatant in step B).

19. The method according to Claim 14, further comprising the step of filtering the material from the step B).

20. The method according to Claim 14, further comprising the step of removing blood cells.

21. The method according to Claim 14 wherein the step of removing blood cells comprises adding a component of degrading blood cells.

22.(Amended) A method for preparing a stem cell, without collagenase treatment, comprising:

i) obtaining material from liposuction;

ii) subjecting the material to a condition where at least a portion of cells are separated from the material, without isolation of fat tissue;

iii) subjecting the material to centrifugation;

iv) adding a component degrading blood cells to the material and agitating the material;

v) subjecting the material to centrifugation to obtain a pellet; and

vi) aspirating supernatant of the material from the pellet.

23. The method according to Claim 22, wherein the step of subjecting the material to said condition comprises maintaining an aspirate from the liposuction.

24. The method according to Claim 22, wherein said material from liposuction comprises an aspirate from liposuction and fat.

(Cancelled)[~~25. The method according to Claim 22, wherein said condition in said step ii) comprises adding a collagenase.~~]

26. The method according to Claim 22, wherein the centrifugation in said step iii) is conducted at 400-1200 x g.

27. The method according to Claim 22, wherein said component degrading blood cells comprises ammonium chloride and potassium bicarbonate.

28.(Amended) The method according to Claim 27, wherein said ammonium chloride is comprised in the component at 155mM.

29. The method according to Claim 27, wherein said potassium bicarbonate is comprised in the component at 10mM.

30. The method according to Claim 22, wherein said centrifugation in said step v) is conducted at 400-1200 x g.

31. The method according to Claim 22, wherein said pellet contains a stem cell.

32. A stem cell prepared by the method according to any of Claims 1-31.

33. The stem cell according to Claim 32, which expresses at least one protein selected from the group consisting of CD13, CD29, CD34, CD36, CD44, CD49d, CD54, CD58, CD71, CD73, CD90, CD105, CD106, CD151 and SH3.

34. The stem cell according to Claim 33, which expresses CD13, CD29, CD34, CD36, CD44, CD49d, CD54, CD58, CD71, CD73, CD90, CD105, CD106, CD151 and SH3.

35. The stem cell according to Claim 33, further expressing at least one protein selected from the group consisting of CD31, CD45, CD117 and CD146.

36. The stem cell according to Claim 32, which does not express CD56.

37. The stem cell according to Claim 32, which does not express at least one protein selected from the group consisting of CD3, CD4, CD14, CD15, CD16, CD19, CD33, CD38, CD56, CD61, CD62e, CD62p, CD69, CD104, CD135 and CD144.

38. The stem cell according to Claim 37, which does not express CD3, CD4, CD14, CD15, CD16, CD19, CD33, CD38, CD56, CD61, CD62e, CD62p, CD69, CD104, CD135 and CD144.

39. The stem cell according to Claim 32, which expresses CD49d and does not express CD56.

40. (Amended) A system for preparing a stem cell, without collagenase treatment, comprising:

A) means for obtaining an aspirate from liposuction;

B) means for subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction; and

C) means for subjecting the cell fraction to centrifugation by specific gravity.

41. The system according to Claim 40, wherein the system further comprises:

D) means for collecting a cell layer with lower specific gravity than that of erythrocytes.

42.(Amended) A system for preparing a stem cell,

without collagenase treatment, comprising:

A) means for obtaining material from liposuction; and

B) means for subjecting the material from liposuction to centrifugation to obtain a cell fraction without isolation of fat tissue.

43.(Amended) A system for preparing a stem cell, without collagenase treatment, comprising:

i) means for obtaining material from liposuction;

ii) means for subjecting the material to a condition where at least a portion of cells are separated from the material, without isolation of fat tissue;

iii) means for subjecting the material to centrifugation;

iv) a component degrading blood cells to the material and agitating the material;

v) means for subjecting the material to centrifugation to obtain a pellet; and

vi) means for aspirating supernatant of the material from the pellet.

44.(Amended) A method for obtaining an explant, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction;

C) subjecting the cell fraction to centrifugation by specific gravity;

D) collecting a cell layer with lower specific gravity than that of erythrocytes;

E) culturing the collected cell layer to obtain an

explant.

45.(Amended) A method for preparing a tissue transplant, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction; and

C) culturing the collected cell layer to obtain a tissue transplant.

46.(Amended) A method for preparing tissue transplant, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction;

C) subjecting the cell fraction to centrifugation by specific gravity;

D) collecting a cell layer with lower specific gravity than that of erythrocytes;

E) culturing the collected cell layer to obtain a tissue transplant.

47.(Amended) A method for transplanting a tissue transplant, without collagenase treatment, comprising:

A) obtaining an aspirate from liposuction;

B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction;

C) subjecting the cell fraction to centrifugation by specific gravity;

D) collecting a cell layer with lower specific gravity than that of erythrocytes;

E) culturing the collected cell layer to obtain a tissue transplant; and

F) transplanting the tissue transplant.

48.(Amended) Use of an aspirate of liposuction in preparing stem cells, without collagenase treatment.

49. A method for preparing cells selected from the group consisting vascular endothelial precursor cells, adipocytes, cartilage cells, bone cells and muscle cells comprising the step of culturing a stem cell

disease, a disorder or an abnormal condition attributed to the deficiency of a differentiated cell, comprising:

a) a stem cell obtained according to any one of Claims 1-31;

b) a differentiated cell corresponding to a desired site; and

c) a pharmaceutically acceptable carrier.

69. Use of a mixture of: a) a stem cell obtained according to any one of Claims 1-31; and b) a differentiated cell corresponding to a desired site, for preparation of a medicament for treatment or prevention of a disease, a disorder or an abnormal condition attributed to the deficiency of a differentiated cell.

70. (Amended) A method for [~~treatment or~~] improvement of a cosmetic condition, comprising the steps of:

A) providing a composition comprising:

a) a stem cell obtained according to any one of Claims 1-26; and

b) a differentiated cell corresponding to a desired site; and

B) administering the composition to a subject.

71. (Amended) A [~~medicament~~] composition for [~~treatment or~~] improvement of a cosmetic condition, comprising:

a) a stem cell obtained according to any one of Claims 1-31;

b) a differentiated cell corresponding to a desired site; and

c) a pharmaceutically acceptable carrier.

72.(Amended) Use of a mixture of: a) a stem cell obtained according to any one of Claims 1-31; and b) a differentiated cell corresponding to a desired site, for preparation of a [~~medicament~~] composition for [~~treatment or~~] improvement of a cosmetic condition.

REPLY

To: Examiner of the Patent Office

1. Identification of the International Application
PCT/JP2004/016717

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4. Date of Notification: 11.07.2005

5. Subject Matter of Correction
5.1 Gist of Response:
The Applicant submits an amendment to amend claims 1, 14, 22, 28, 40, 42-48 and 70-72; delete claims 16, 17, and 25;

and remove the following description from the specification (corresponding to WO 2005/042370A2): page 7, lines 15-19; page 8, lines 23-25; page 25, portions of lines 5, 6, 26 and 27; page 32, lines 12-15; and page 61, lines 8 and 9. The Applicant also submits argument to assert that the present application complies with Rule 6.3(a) PCT, Rule 13 PCT, Article 33(2) PCT and Article 33(3) PCT.

5.2 Summary of Amendment:
The Applicant amended claims 1, 14, 22, 40 and 42-48 to include the recitation "without collagenase treatment".

The Applicant amended claim 70 to recite "A method for [~~treatment or~~] improvement...".

The Applicant amended claim 71 to recite "A [~~medicament~~] composition for [~~treatment or~~] improvement...".

The Applicant amended claim 72 to recite "...a [~~medicament~~] composition for [~~treatment or~~] improvement...".

The Applicant amended claim 28 to correct a typographical error.

The Applicant deleted claims 16, 17 and 25.

The Applicant deleted the following description from the specification (corresponding to WO 2005/042370A2): page 7, lines 15-19; page 8, lines 23-25; page 25, portions of lines 5, 6, 26 and 27; page 32, lines 12-15; and page 61, lines 8 and 9, which refer to collagenase treatment as a preferred embodiment of the present application.

5.3 Clarification of the unity and inventive step of the invention:

Re Item IV

In the Amendment, the Applicant has amended the independent claims to include the recitation "without collagenase treatment". This recitation, which is supported by Example 2 of the WO 2005/042370A2 specification, reflects a significant technical feature of the present invention. Therefore, the present application complies with Rule 6.3(a) PCT.

The Applicant argues that the amendment to the independent claims, to recite the technical feature "without collagenase treatment", confers unity to the amended claims. Therefore, the present application complies with Rule 13 PCT.

Item V

The Applicant has amended the independent claims of the present application to recite the significant technical feature of "without collagenase treatment". The Applicant argues that this technical feature confers a surprisingly significant effect to the present invention.

Unexpectedly, omitting the use of collagenase, which is used in conventional techniques to separate fat tissue from the starting liposuction material, produced an improved yield of a population of surprisingly good quality, homogeneous, adipose-derived stem cells. The added significant advantage being that the method is simple and works in a surprisingly efficient manner.

Cited References D1-D5 neither teach nor suggest the preparation of adipose-derived stem cells from liposuction material without prior collagenase treatment. One skilled in the art would not expect to obtain adipose-derived stem cells of such high quality and with such improved yield from a starting material not treated with collagenase by such an advantageously simple and efficient method. Surprisingly, when the stem cells purified by the present invention are compared to those prepared by conventional methods, the resulting stem cell populations exhibit similar phenotypes (compare Examples 3 and 15 of WO 2005/042370A2). Hence, the recitation "without collagenase treatment" in the independent claims, renders the invention of the amended claims, and dependent claims therefrom, novel and inventive in view of the disclosure of Cited References D1-D5. Therefore, the present application complies with Article 33(2) PCT and Article 33(3) PCT.

Re Item VIII

The Applicant has amended claims 70-72 to clarify that the amended claims are directed to methods and compositions for the improvement of a cosmetic condition by removing the recitations "treatment" and "medicament".

Regarding the references to embryonic stem cells (page 25, lines 5 and 26, corresponding to WO 2005/042370A2) and plant tissues (page 32, lines 12-15, corresponding to WO 2005/042370A2), the Applicant has removed the above referenced descriptions to avoid confusion.

The described protocol in the paragraph bridging pages 54 and 55 of the WO 2005/042370A2 specification, represents

conventional protocols described in the prior art according to the references listed on page 54, lines 20-23 of the WO 2005/042370A2 specification.

5.4 Conclusion:

For the reasons described above, after amendment the present application complies with Rule 6.3(a) PCT, Rule 13 PCT, Article 33(2) PCT and Article 33(3) PCT.